LIMNOLOGY
and
OCEANOGRAPHY: METHODS

Limnol. Oceanogr.: Methods 12, 2014, 1–9

Determination of monomethylmercury from seawater with ascorbic acid-assisted direct ethylation

Kathleen M. Munson[1], Diana Babi[2], and Carl H. Lamborg[1]*
[1]Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, Woods Hole, MA, 02543 USA
[2]Tekran Instruments Corporation, 330 Nantucket Boulevard, Toronto, Ontario, M1P 2P4, Canada

Abstract

We developed a technique to measure monomethylmercury (MMHg) concentrations from small volumes (180 mL) of seawater at low femtomolar concentrations using direct ethylation derivitization, decreasing the required volume by 90% from current methods while maintaining a 5 fM detection limit. In this method, addition of ascorbic acid before derivitization of MMHg allows for full recovery of MMHg from the seawater matrix without the need for sample distillation or extraction. The small sample size and relative ease of detection are ideal both for shipboard as well as shore-based measurements of preserved MMHg samples. Combined with shipboard determination of dimethylmercury (DMHg) and elemental mercury (Hg(0)), this method can be used to determine full marine mercury speciation.

Mercury (Hg) is a toxic metal with an organic monomethylmercury (MMHg) chemical form that bioaccumulates in aquatic food webs. At sufficiently high concentrations, MMHg toxicity can cause decreased fertility and offspring survival in the upper trophic levels of terrestrial and marine systems (Scheuhammer et al. 2007). In humans, MMHg acts as a neurotoxin and can cause developmental defects to fetuses and small children (Clarkson and Magos 2006). Due to the importance of marine protein sources in human diets, efforts to improve our understanding of the production and distribution of MMHg in open-ocean systems have increased in recent decades. However, until recently, descriptions of Hg cycling in marine systems have relied heavily on studies of coastal and sedimentary systems, in which higher MMHg concentrations are more easily measured.

Determination of monomethylmercury concentrations is analytically challenging due to its femtamolar concentrations in much of the open ocean (Cossa et al. 2011; Hammerschmidt and Bowman 2012; Mason and Fitzgerald 1993; Sunderland et al. 2009). Analytical methods, based on ethylation, to quantify MMHg, including the US EPA Standard Method 1630, require separation of MMHg from its environmental matrix. Either solvent extraction (Bloom 1989; Horvat et al. 1993) or distillation (Horvat et al. 1993) methods are commonly used to isolate MMHg for analysis. Following separation, MMHg is volatilized through derivitizaton, most commonly by addition of the ethylating agent sodium tetraethylborate (NaTEB) to form gaseous methylethylHg. Preconcentration of gaseous methylethylHg is achieved through trapping onto a Carbotrap (Bloom 1989) or Tenax (Bowman and Hammerschmidt 2011) column before analysis by GC separation of the methylethylHg and gaseous diethylHg produced from Hg(II) substrate in solution. Once preconcentrated, sub-pM concentrations of MMHg can be quantified by atomic fluorescence spectrometry (AFS) or isotope dilution inductively-coupled mass plasma spectrometry (ID-ICPMS).

Initial attempts to avoid either MMHg distillation or extraction steps by direct ethylation of MMHg with NaTEB were inefficient, recovering 5% to 60% of added MMHg (Horvat et al. 1993). However, a method has recently been developed to detect MMHg from direct ethylation of 2 L volumes of seawater (Bowman and Hammerschmidt 2011). This method is advantageous for two reasons. First, it requires a minimal amount of sample processing compared with MMHg distillation or extraction, which makes the technique amenable for ship-based analyses. Second, the method allows for quantification of both dissolved MMHg and the gaseous dimethylmercury (DMHg) organomercuric chemical species in seawater. The 2-L sample bottles can be purged with nitrogen (N_2) gas to measure DMHg concentrations in seawater before acidification and ethylation to volatilize MMHg. However, the 2-L sample volume can hinder shore-based analysis due to difficulties in sample transport and storage for high-resolution

*Corresponding author: E-mail: kathleenmunson@gmail.com

Acknowledgments

This research was supported by a National Science Foundation grant (OCE-1031271) awarded to C. H. L. and Mak Saito and a Graduate Research Fellowship awarded to K. M. M.

DOI 10.4319/lom.2014.12.1

depth profiles. In addition, the efficiency of direct ethylation from seawater is variable, requiring careful consideration of appropriate standards for MMHg quantification (Bowman and Hammerschmidt 2011).

Before development of the direct ethylation method, preservation of samples with acid for shore-based MMHg determination using distillation or extraction methods prevented separate determination of DMHg and MMHg since DMHg decomposes to MMHg within a matter of hours in acidic conditions (Parker and Bloom 2005). As a result, profiles of acid-preserved samples cannot distinguish between the DMHg and MMHg organomercuric forms in their analyses and instead represent a combined ([DMHg + MMHg]) concentration (e.g., Sunderland et al. 2009).

Direct volatilization is also possible from hydride generation of mercury hydrides via the addition of sodium tetrahydridoborate (sodium borohydride) followed by collection on a cold-trap before analysis using AFS (Cossa et al. 2009). Like other methods, hydride generation requires application at sea to distinguish between DMHg and MMHg. Otherwise, quantification of acid-preserved samples yields combined ([DMHg] + [MMHg]) concentrations (Cossa et al. 2011). However, liquid N_2 is required to analyze the volatile mercury hydride compounds. In addition, like the 2-L direct ethylation method, the mercury hydride method cannot be easily automated or adapted to in-line analysis for isotopic studies.

To address the limitations of current methods, we developed a method that improves the recently described direct ethylation method while simultaneously allowing for sample preservation. Ascorbic acid addition has been found to improve MMHg detection from distilled samples using US EPA Method 1630 (Tekran Instruments Corporation 2011). We therefore adapted the use of ascorbic acid to improve the low ethylation efficiency of MMHg from seawater samples observed using the direct ethylation method (Bowman and Hammerschmidt 2011). The method can be coupled with purging prior to MMHg acidification and derivatization to collect and quantify volatile DMHg as a distinct species (Lamborg et al. 2012).

Materials and procedures

Plastic and glassware

All plastic and glass bottles, tubing, and filter holders for water collection, reagents, and sample preparation were acid-washed in a class 100 clean room according to described protocols (Hammerschmidt et al. 2011). Pre-filters for water filtration were combusted prior to use. Capsule filters were filled with 10% HCl, soaked for 24 h, and rinsed with de-ionized water (>18 MΩ cm; "MQ") until the rinse water pH was > 6.

Either 42-mL amber glass vials (I-CHEM, Fisher) or 250-mL amber glass bottles (I-CHEM, Fisher) were used for MMHg analysis from 30-mL or 180-mL sample volumes, respectively. The sample volumes were chosen to maximize the MMHg signal detected from the purged sample while maintaining adequate headspace for appropriate positioning of the instrument's dual sample purge and analyte inlet needle (see below) above the height of the liquid. New Teflon backed septa (SunChem) were soaked in Citranox (1%) overnight and 10% HCl for > 24 h, then rinsed with MQ prior to use. Previously pierced septa were reused after soaking in Citranox (1%) overnight and up to 6 days in 10% HCl and rinsed with MQ before use.

Seawater

Seawater used for method development was collected from the Equatorial Pacific Ocean in October 2011 on board the R/V *Kilo Moana* and from Vineyard Sound in Woods Hole, Mass., USA, intermittently between November 2011 and March 2012. Open-ocean seawater was collected in acid-rinsed X-Niskin bottles on a dedicated trace-metal clean rosette deployed on Amsteel line. Seawater was filtered (47mm, Supor polycarbonate membrane, 0.2 μm pore size, Pall Corp.) from X-Niskin bottles that were pressurized with ultra high purity N_2 in a positive pressure, HEPA-filtered bubble constructed onboard the ship. Water was decanted into acid-clean 10-L polyethylene carboys and stored at room temperature until use. Vineyard Sound coastal seawater was collected in acid-cleaned 2-L Teflon bottles mounted on a pole sampler from the shore at the Quissett Campus of the Woods Hole Oceanographic Institution. Water was filtered with a GF/A pre-filter (42-mm, Whatman) and a 0.2-μm Sterivex-GV (Millipore) filter.

Monomethylmercury determination

MMHg was determined by CVAFS gas chromatography with a Tekran 2700 Automated Methyl Mercury Analysis System (Tekran, Ontario, Canada). The system is designed to determine MMHg from 30 mL samples of freshwater. Samples were analyzed using an autosampler that features a septa-piercing needle equipped with a purge gas outlet and a sample inlet. After piercing the septum, Ar flow through the tip of the needle purges the sample and allows the loading of gaseous methylethylHg through the sample inlet until the needle withdraws from the sample vial. The dual purging and loading allows for in-line loading of the Tenax column (Supelco) and separation of derivitized Hg species through a GC column.

In the manufacturer set-up of the instrument, an event table file is used to automate sample purging followed by loading and heating of the Tenax trap and GC column via a sequence of valve activation and deactivation. Gaseous species of Hg are purged from the sample at controlled flow rates (82, 120, or 152 mL/min) and loaded onto a Tenax trap at 31°C while bypassing the GC column, which has a separate continuous flow of Ar. During the desorption state, the valve between the Tenax trap and the GC column is opened, the Tenax trap is heated to 180°C, and the desorbed Hg derivatives are carried from the Tenax trap to the GC column (85°C). The chromatographically separated mercury species are fully combusted in a quartz pyrolysis column (720°C), combined with an Ar make up gas, and quantified by AFS. The Tenax trap and GC column are heated between sample loadings to avoid carryover between samples. The resulting chromatograph dis-

plays separate peaks of Hg(0), methylethylHg, and diethylHg, of which the methylethylHg peak can be quantitatively integrated to determine MMHg concentration.

Fixed volumes (± 1 mL, determined gravimetrically) were used for both standard curve and sample preparation. The maintenance of a consistent headspace was necessary to produce linear standard curves (Fig. 1). As a result of the fixed volumes, the calibrated sample peak areas could be rendered in concentration units.

Modifications of factory-installed software and hardware were necessary to detect MMHg from 180 mL seawater using the Tekran 2700. The autosampler comes equipped with 3 trays that each hold 21 42-mL sample vials that correspond to pre-set autosampler positions. Although the autosampler has alternative settings for smaller sample vial sizes, there is no pre-installed set-up for sufficiently large volume samples for seawater detection. Therefore, a custom foam and cardboard tray that accommodates 250-mL volume bottles was designed for bottle positions that correspond to vial positions that are pre-set for the autosampler configuration for 3 trays of 40 sample vials. To accommodate the differences in height between the 42-mL and 250-mL sample bottles, the z-axis height setting of the dual purge/inlet needle was adjusted to keep the sampling inlet in the headspace during sample loading (from 14700 mm to 13400 mm in the manufacturer autosampler configuration). In addition, the vial stopper was adjusted to allow clearance over the height of the bottles.

To maximize the signal, the flow rate of Ar gas for sample purging and the sample purging time were increased. The Ar gas flow was modified from manufacturer's installation by replacing the original 82-mL/min flow restrictor with a 152-mL/min flow restrictor. In addition, a new event table file was created that extended the sample purge time from 4 min to 8 min. As with the recommended configuration for analysis of 30-mL samples of freshwater, the gas stripping of MMHg from the 180 mL samples is not complete despite the extended purge time, but approaches 80% after 8 min. This extended purge time was chosen as a balance between maintaining the shortest possible analysis time, minimizing the amount of moisture that is forced onto the Tenax column and maximizing the MMHg signal.

Standard and sample preparation

Acetate and citrate buffers, potassium hydroxide, and sulfuric acid were stored in acid-cleaned Teflon bottles. Solutions were remade as needed and to address any contaminants detected from reagent blanks.

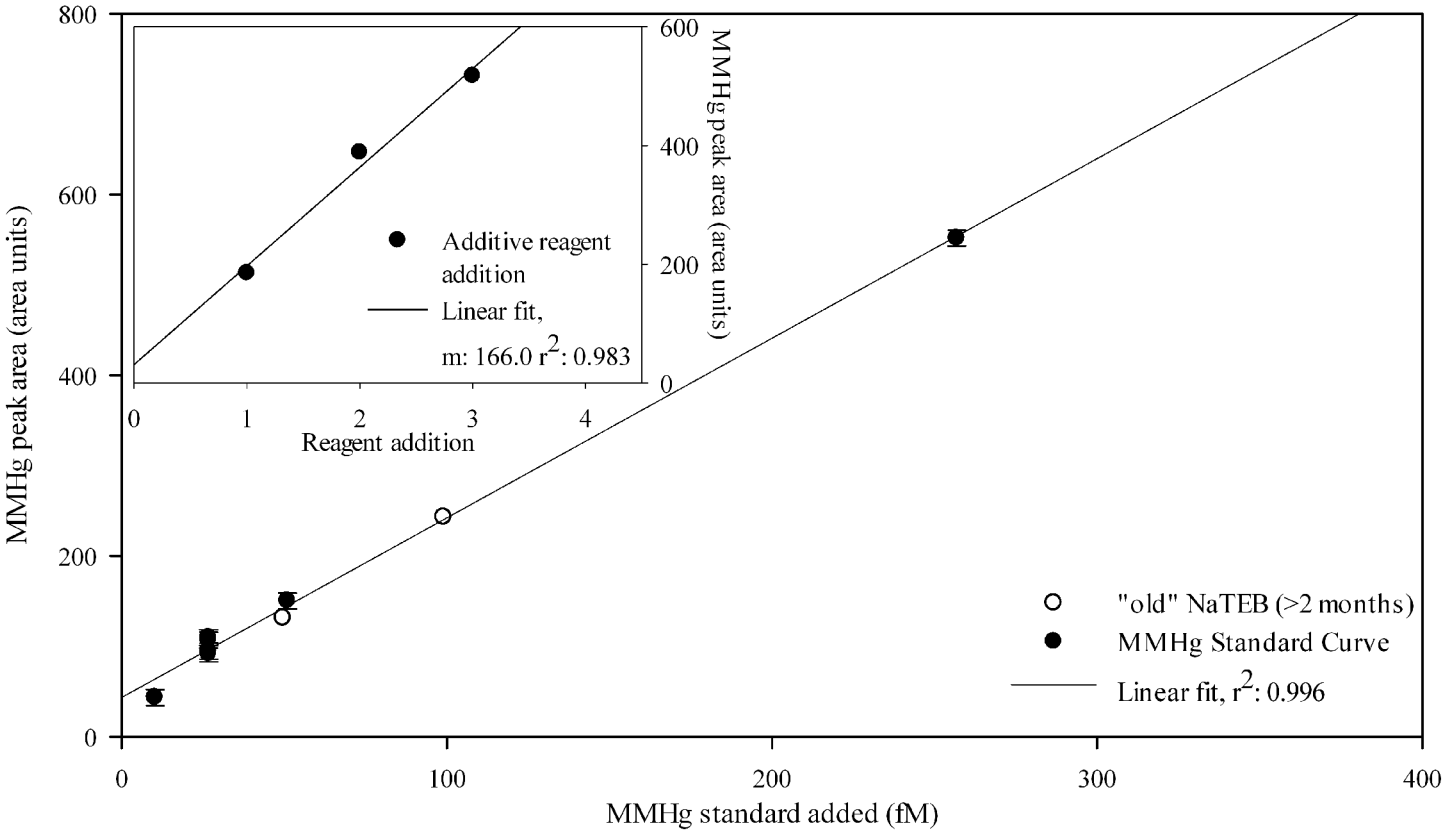

Fig. 1. Representative standard curve of instrument MMHg peak area versus added MMHg standard (15-250 fM) from 180 mLvolumes used to quantify seawater samples. Four replicates of 25 fM are shown and are used to calculate standard variability. Inset: MMHg blank in reagents is calculated from the slope of the linear fit obtained from the instrument MMHg peak area versus stepwise increases in reagent concentrations.

Table 1. Reagents optimized for 30 mL and 180 mL sample volumes. Acetate buffer (2M) was used initially as outlined in USEPA Standard Method 1630 (2001). However, citrate buffer (1M) replaced acetate buffer for low concentration seawater samples to eliminate ingrowth of MMHg in samples upon repeated analytical runs of the individual samples.

Final sample volume (mL)	Detection limit (fM)	Ascorbic acid (μL)	2M acetate buffer (mL)	1M citrate buffer (mL)	NaTEB (μL)
30	100	50	0.225	0.55	30
180	5	300	1.0	2.0	85

For standard curve preparation, secondary stocks of 50 pM were prepared from dilution of concentrated MMHg primary stock (Alfa Aesar, 1000 ppm methylmercury chloride in water) in MQ and acidified to 0.25% HCl (concentrated, SEASTAR Chemical) to prevent loss of analyte to sides of the glass bottle. Standard curves ranging from 10 fM to 1000 fM were prepared daily from the secondary stock in MQ, filtered Equatorial Pacific seawater, and filtered Vineyard Sound seawater. MMHg was equilibrated with ambient ligands for at least 24 h before further processing.

After equilibration, all samples and standards were acidified to a final concentration of 0.5% with concentrated H_2SO_4 (Fisher, Trace Metal Grade) to extract MMHg from the seawater matrix and allowed to react for 24 h before ethylation (Bowman and Hammerschmidt 2011). Ascorbic acid (2.5% wt:vol in MQ, Fisher) was added to each sample prior to ethylation. Sample pH was then adjusted to 5 with KOH (45% wt:vol in MQ, Fisher). Sample pH was determined by testing 20 μL volumes of sample on pH strips (MColorpHast range 4.0-7.0, EMD Millipore). Neutralized samples were buffered with either acetate buffer (2M sodium acetate, Fisher; adjusted to pH 5 with H_2SO_4, conc. Fisher) or citrate buffer (1M sodium citrate Fisher; adjusted to pH 5 with H_2SO_4, conc. Fisher) according to sample size (Table 1). Sodium tetraethylborate (NaTEB, Strem Chemical) was dissolved (1% final wt:vol) in prechilled 2% KOH in MQ and quickly aliquoted into 10-mL Teflon vials and kept frozen until use. For sample ethylation, an aliquot of NaTEB was thawed just until it formed a slush and was added to the buffered sample. Samples were then capped with Teflon-backed septa and inverted 10 times. The ethylation reaction was allowed to proceed for at least 20 min before initiating analysis.

Sample bottles were loaded into either the 42-mL vial tray provided by the manufacturer or the custom 250-mL bottle foam tray. Samples were analyzed after standards showed a linear relationship and peak area values for standards could be distinguished from those of reagent blanks. Since the custom tray accommodates a maximum of 11 sample bottles, sets of 8-11 samples were typically prepared, and all were analyzed within 4 h of ethylation.

Assessment

The development of the presented method was focused on achieving two goals. First, we attempted to lower the detection limit of the automated method to adequately measure the low femtomolar concentrations of MMHg in open-ocean water. We did this by increasing the total sample volume from 30-mL to 180-mL as well as increasing total purge flow for larger sample volume by lengthening the purge time as well as increasing the Ar purge rate.

Second, we sought to improve the efficiency of MMHg extraction from the saltwater matrix. This was accomplished by the addition of ascorbic acid, which allowed for full extraction of added MMHg from seawater. We also replaced the acetate buffer commonly used in MMHg determination methods with citrate buffer to reduce signal inconsistencies that we observed after making alterations to the instrument method.

Sample volume adjustment

Using the instrument as designed with 30-mL sample volumes of seawater, the detection limit of 100 fM, as calculated from 3× the standard deviation of the blanks, was insufficient for low open-ocean MMHg concentrations, typically < 100 fM in the upper water column (Cossa et al. 2011; Hammerschmidt and Bowman 2012; Mason and Fitzgerald 1993; Sunderland et al. 2009). The modification of the instrument to accommodate an increase in sample volume to 180-mL resulted in a daily detection limit of ~ 5 fM, from triplicate reagent blanks, which was sufficient for most depths within the open-ocean water column. This detection limit is on the same order of magnitude as that of the recently described 2-L direct ethylation method (Bowman and Hammerschmidt 2011) while decreasing the required volume by 90% and allowing the process to be automated.

Ar purge rate and purge volume

Unlike alternative analytical methods, the Tekran automated instrument used in this study does not completely purge MMHg from the sample. Instead, a purge time of up to 4 min is recommended for MMHg determination from freshwater samples for adequate mercury species separation. However, we observed a methylation artifact when using a 4 min purge time with 180 mL sample volumes. This artifact appeared after repeated analyses of individual acetate buffered samples and produced MMHg signals ranging between 73% to 477% (n = 5) of the initial MMHg signal (Fig. 2A). The artifact was most pronounced in samples and standards with MMHg concentrations near 15 fM (Fig. 2B, C). We increased the purge time to 8 min to extract a higher proportion of the MMHg present in samples. The increased purge time resulted in a predictable decrease in measured MMHg from the first purge to the second but not in subsequent purges of an individual standard buffered with acetate

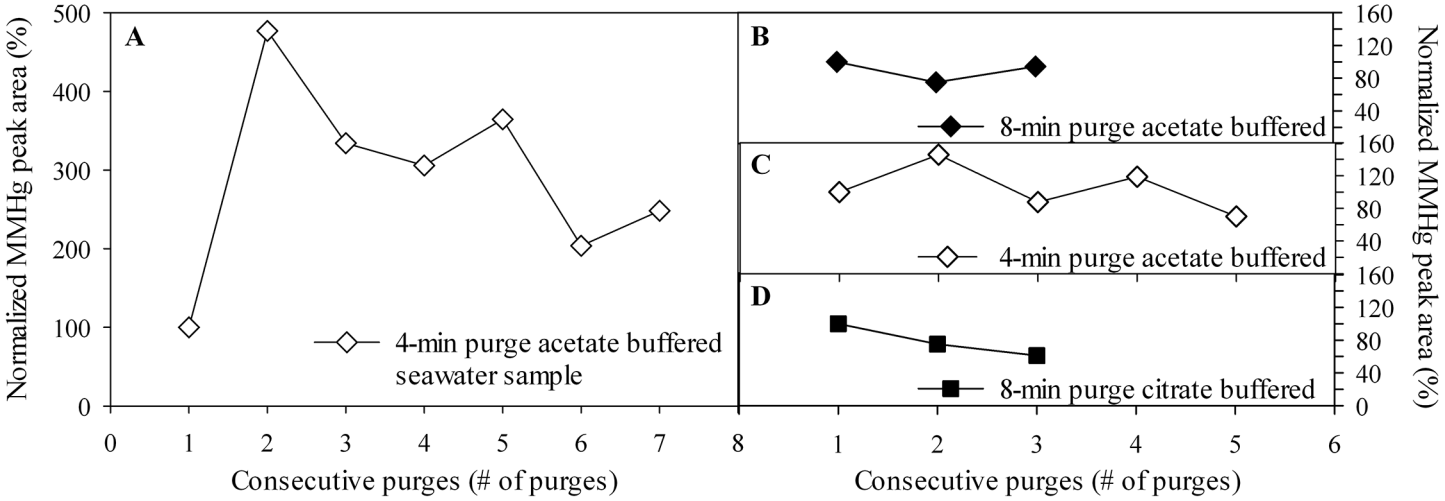

Fig. 2. Increases in measured MMHg, relative to the initial MMHg measured, were observed during repeated analysis of individual seawater samples prepared with acetate buffer (180 mL) (A). Extending the Ar purge time for acetate-buffered standards to 8 min failed to alleviate this variability (B). Similar variability was observed in acetate-buffered standards of 25 fM MMHg added to filtered seawater using a 4-min Ar purge (C). Quantitative stripping of MMHg was observed from citrate-buffered standards using an 8-min Ar purge (D).

(Fig. 2B). It is important to note that this artifact was not the result of MMHg carry over from previously analyzed samples. Concentrations of MMHg measured from blanks run between repeated purging of individual samples or standards were not elevated (<DL). Because acetate buffers are known to produce MMHg when exposed to light (e.g., Falter 1999), we sought to avoid the apparent MMHg production in acetate buffered samples by using citrate buffer (Fig. 2D, see below).

Although we increased both the Ar purge rate and the Ar purging time, the increase in the Ar purge rate from 80 mL/min to 120 mL/min produced only a modest increase in the MMHg sample signal of ~ 5% (n = 5) from 30-60 mL sample volumes. In contrast, the increase in total purge time from 1 min 45 s to 4 min increased the MMHg sample signal by 25% (n = 5). The failure of increases in the purge rate to increase sample signal may be attributed to the increase in gas pressure in the headspace above the aqueous sample, and therefore, a decrease in the volatilization of methylethylHg from the aqueous sample. Although the MMHg signal was less sensitive to increases in the Ar purge rate than to increases in the sample volume, the combined increase in purge rate and length of purge allowed for samples to be processed in a relatively high throughput manner while still retaining low femtamolar detection limits.

Determination of reagent blanks

Due to low MMHg concentrations present in open-ocean seawater, it is important to quantify the Hg species content of reagent blanks and take steps to minimize contamination. MQ in our laboratory often has measurable MMHg and Hg(II) concentrations that would hinder analysis of low seawater concentrations. As a result, we used two methods to quantify MMHg in the reagent blanks. First, MQ was exposed to ambient outdoor UV in 2-L Teflon bottles for 7-10 d until the concentration of MMHg in the water was negligible (<3 fM), and this water was then used to prepare reagent blanks. Alternatively, to check the MMHg contribution of the reagents to the total signal from blanks, the reagents prepared in lab MQ were added in increasing amounts (e.g., 1×, 2×, and 3× above those values in Table 1) and were determined as the MMHg signal above the MQ water background (Fig. 1).

The precision of the standard curves was determined daily to have an average relative standard deviation from 4 replicate standards of 6% (range: 4% to 9%) during method development. Generally, the slope of the standard curve was also consistent from day to day. Over a 14-day period, the average slope of daily standard curves of MMHg peak area versus the amount of MMHg added (fM) averaged 2.61 (± 0.31) area units per fM Hg for 180 mL volume standards. Differences in the slopes between standard curves prepared with MQ and those prepared with filtered seawater were similar, varying between –12% to + 16% (n = 3; Fig. 3) for 180 mL volumes without the need to adjust for reduced ethylation efficiency due to the seawater matrix. As a result, standard curves prepared in MQ can be used for quantification of MMHg concentrations.

Ascorbic acid addition

The addition of ascorbic acid dramatically improved the extraction of MMHg from the seawater matrix. Extraction of MMHg standard from filtered seawater averaged 34% (range: 2.7% to 81%, n = 10) and improved to 97% (range: 86% to 109%, n = 7) when ascorbic acid was added (Fig. 4). Although the mechanism by which MMHg recovery was improved is unknown, the function of ascorbic acid to overcome seawater matrix effects is affected by neither the order of reagent addition nor the length of time that ascorbic acid is allowed to

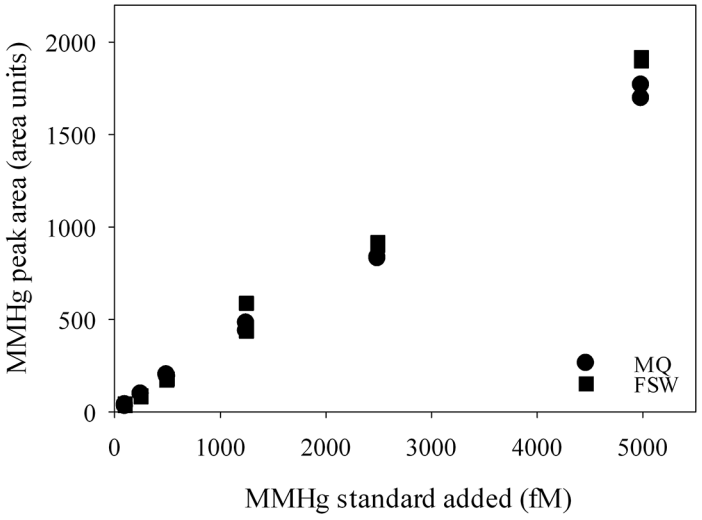

Fig. 3. Recovery of added MMHg standard from de-ionized water (MQ) and filtered Vineyard Sound seawater (FSW) using ascorbic acid assisted direct ethylation. Instrument MMHg peak areas are corrected for reagent blanks measuring from each of the corresponding sample matrices. The addition of ascorbic acid to the FSW prevents the need for correcting for low ethylation efficiency from direct ethylation of seawater. Although open-ocean concentrations of MMHg are generally well below 1000 fM, standards show similar recovery from FSW and MQ beyond the range required for determining ocean MMHg concentrations.

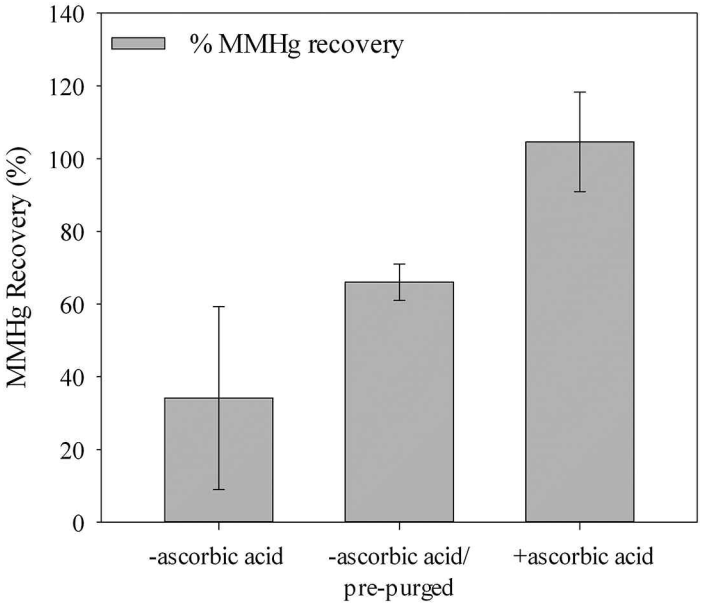

Fig. 4. Percent recovery of added MMHg standard (0.02-1ppt) equilibrated for 24 h in filtered Vineyard Sound seawater measured by direct ethylation. In the absence of ascorbic acid (left) the recovery of MMHg is generally less than 40%. Purging of filtered seawater before MMHg standard addition (center) may increase the recovery. The addition of ascorbic acid (right) yields significantly greater recovery from seawater not purged before standard addition ($P < 0.005$).

react with the seawater sample. The increased extraction efficiency rendered by ascorbic acid yielded 98% recovery of equilibrated MMHg standard spikes from 30-mL volumes of filtered seawater within the 20-min reaction time allowed for the ethylation reaction (range: 81% to 128%, $n = 15$). Extending the reaction time by first adding ascorbic acid, resealing the sample bottle, incubating for 12 h, and then adding the remaining reagents yielded slightly higher recoveries, 130% (range: 104% to 155%, $n = 8$), of added MMHg standard. In addition, recovery of MMHg from previously purged filtered seawater to which no ascorbic acid was added was enhanced, from 34% to 70% (range: 61% to 71%, $n = 2$) compared with unpurged filtered seawater (Fig. 4). This suggests that a volatile, oxidizing component of seawater may be the source of interference that inhibits extraction of MMHg from the seawater matrix.

Addition of ascorbic acid to samples before ethylation also greatly improved the effective life span of NaTEB. Previous studies have found that NaTEB quickly loses its ethylation ability in a matter of days, even when kept frozen in individual aliquots until use (USEPA 2001; DeWild et al. 2002; Lamborg et al. 2012). The addition of ascorbic acid extended the ethylation capacity of NaTEB. Individual aliquots of NaTEB were stable through several freeze and thaw cycles over weeks of analyses without noticeable changes in the ability to ethylate MMHg standards. Dissolved NaTEB stored frozen (–20°C) for > 2 months maintained its ability to ethylate MMHg equilibrated in filtered seawater (~ 88% recovery of added MMHg; $n = 2$; Fig. 1). This suggests that the perceived instability of NaTEB is not due to chemical breakdown from oxidation during storage, but is instead a result of interferences with the ethylation reaction by some component of either the seawater matrix or a breakdown product of NaTEB itself.

Citrate buffering

Contamination from reagents can be minimized through the use of trace metal grade acids and bases. However, the organic acid buffers can contribute a significant amount of MMHg contamination to sample signals. In initial attempts to reduce this contamination, NaTEB was added to the acetate buffer, allowed to react at room temperature for 20 min, and purged with Hg-free N_2 at 0.5-L/min for 30 min to remove MMHg and inorganic Hg species. However, upon analysis, it was discovered that this daily addition of NaTEB and purging of the acetate buffer contributed to an in-growth of MMHg observed with subsequent purges of individual samples/standards (Fig. 2). As a result, we replaced the acetate buffer with citrate buffer to avoid similar production of MMHg during analysis. Although the citrate buffer has a higher concentration of MMHg contamination, ranging between 5 and 73 fM ($n = 15$) compared with less than 5 fM in acetate buffer, the contamination from the citrate buffer did not appear to increase with time or exposure to light. Repeated purging of citrate buffered samples for 8 min also resulted in a predictable decrease in the MMHg peak area averaging 77% of its previous

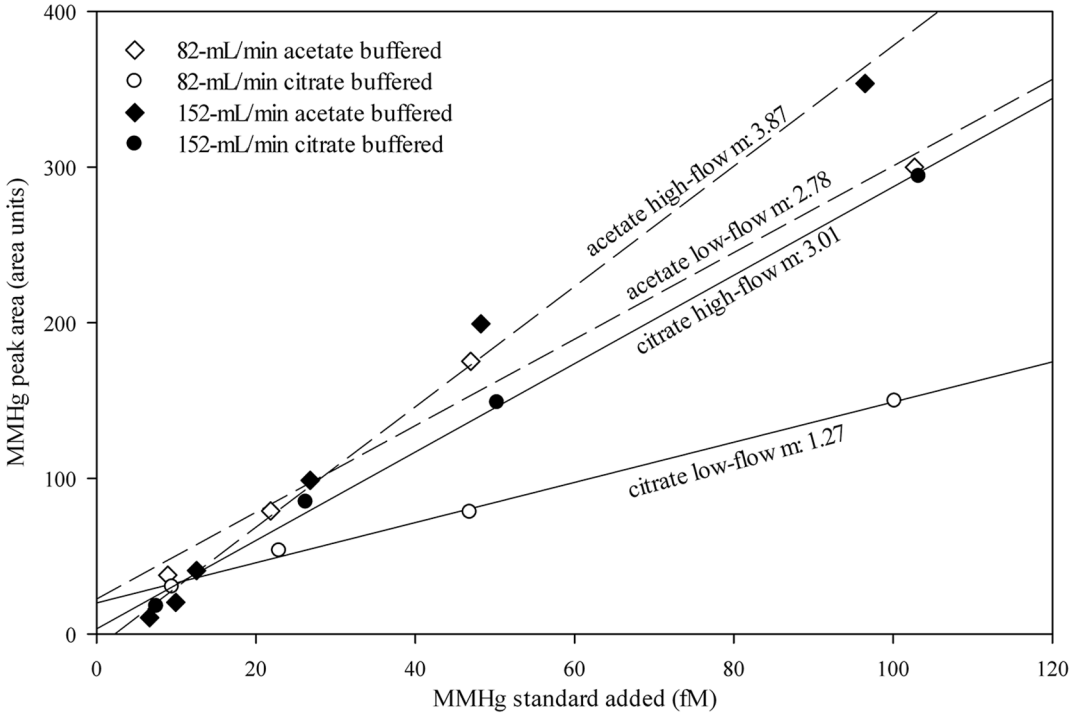

Fig. 5. Comparison of standard curves of instrument MMHg peak area versus added MMHg standard (5-100 fM) prepared with acetate buffer and citrate buffer. Citrate buffered standards yield less sensitivity as observed from the lower slope of the standard curve. By increasing the rate of the Ar purging during sample analysis by replacing the 82-mL/min flow restrictor with a 152-mL/min flow restrictor, the sensitivity of the citrate-buffered standard curve approaches that of the acetate-buffered standard curve.

value (range: 74% to 82%, $n = 6$) without the MMHg in-growth observed with acetate buffered MMHg concentrations < 15 fM.

In addition to the greater MMHg contamination found with citrate buffer, its use decreased the sensitivity of the MMHg detection, calculated from the slope of the MMHg peak area versus concentration of added MMHg standard, to 45% that of acetate buffered standards at a flow rate of 82 mL/min. However, at the higher 152 mL/min flow rate, the slope of the citrate buffered standards is 77% that of acetate buffered standards (Fig. 5).

Discussion

Direct ethylation of MMHg has provided a means to vastly improve the understanding of Hg speciation in marine systems, especially the organomercuric species DMHg and MMHg, which are present in low femtomolar concentrations in the open-ocean water column (Cossa et al. 2011; Hammerschmidt and Bowman 2012; Mason and Fitzgerald 1993; Sunderland et al. 2009). Because sample preservation with acid leads to the demethylation of DMHg to MMHg (Parker and Bloom 2005), a method capable of distinguishing between these two organomercuric species is essential to understand the partitioning of Hg species. Although the direct ethylation method provides a promising alternative to sample distillation, the seawater matrix presents interferences that can lower ethylation efficiency.

Although the addition of ascorbic acid does not lower the detection limit (~5 fM) compared with the current method of direct ethylation of MMHg from sweater (Bowman and Hammerschmidt 2011), complete recovery of MMHg from the seawater matrix is advantageous for two reasons. First, the ethylation enhancement observed using ascorbic acid, presumably due to its function as a mild reductant, may occur to unknown degrees in seawater samples, depending on their concentrations of naturally occurring reductants. As a result, without complete MMHg extraction, relative concentrations between seawater samples may reflect differences in ethylation efficiency due to the composition of the seawater matrices rather than differences in MMHg concentrations. Second, the increased ethylation efficiency observed in pre-purged seawater (Fig. 4) can potentially overestimate the assumed ethylation efficiency when MMHg standard curves are prepared in previously purged seawater (Bowman and Hammerschmidt 2011).

Preservation of open-ocean seawater samples can be essential for mechanistic studies of mercury species cycling in ocean environments. Thus, the presented ascorbic acid-assisted direct ethylation method has been developed to allow for both ship-based and lab-based MMHg measurement. In combination with ship-based measurement of DMHg from untreated seawater prior to preservation with H_2SO_4, this method overcomes major restrictions faced while using many of the current methods used for MMHg analysis. In addition,

our semi-automated method can improve throughput for linked analyses, such as CVAFS-ICPMS for mechanistic studies using additions of stable Hg isotopes (Monperrus et al. 2007; Lehnherr et al. 2011).

Comments and recommendations

We developed the described method specifically to reduce the required volume needed to measure MMHg from seawater while maintaining a minimal amount of sample manipulation. The addition of ascorbic acid enables complete extraction of low open-ocean concentrations of MMHg from seawater matrices. Complete extraction is important for avoiding potential biases in MMHg determination that may result from changes in ethylation efficiency caused by differences in seawater matrix composition between samples.

Direct methylation from Hg(II) has recently been revealed as a potentially significant source of MMHg in the marine water column (Lehnherr et al. 2011; Sunderland et al. 2009). However, MMHg may also be produced from the abiotic degradation of highly photolabile DMHg (Mason et al. 1995). Because we developed this method to measure MMHg from acid-preserved samples that were stripped of DMHg before preservation, we have not run standard curves to quantify the DMHg detected by the Tekran 2700. Thus, we recommend that the described method be used in conjunction with ship-based measurements of DMHg for full dissolved Hg speciation analysis (Bowman and Hammerschmidt 2011; Lamborg et al. 2012).

Although our method was developed using one of two commonly used commercially available MMHg auto analyzers, the addition of ascorbic acid can be adapted to existing manual methods to minimize matrix effects during analysis.

References

Bowman, K. L., and C. R. Hammerschmidt. 2011. Extraction of monomethylmercury from seawater for low-femtomolar determination. Limnol. Oceanogr. Methods 9:121-128 [doi:10.4319/lom.2011.9.121].

Bloom, N. 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Can. J. Fish. Aquat. Sci. 46, 1131-1140 [doi:10.1139/f89-147].

Clarkson, T. W., and L. Magos. 2006. The toxicology of mercury and its chemical compounds. Crit. Rev. Toxicol. 36:609-662 [doi:10.1080/10408440600845619].

Cossa, D., B. Averty, and N. Pirrone. 2009. The origin of methylmercury in open Mediterranean waters. Limnol. Oceanogr. 54:837-844 [doi:10.4319/lo.2009.54.3.0837].

———, and others. 2011. Mercury in the Southern Ocean. Geochim. Cosmochim. Acta 75:4037-4052 [doi:10.1016/j.gca.2011.05.001].

DeWild, J., M. Olson, and S. Olund. 2002. Determination of methylmercury by aqueous phase ethylation, followed by gas chromatographic separation with cold vapor atomic fluorescence detection. U. S. Geol Surv. Open File Rep 01-445.

Falter, R. 1999. Experimental study on the unintentional abiotic methylation of inorganic mercury during analysis. Part 2: Controlled laboratory experiments to elucidate the mechanism and critical discussion of the species specific isotope addition correction method. Chemosphere 39:1075-1091 [doi:10.1016/S0045-6535(99)00179-4].

Hammerschmidt, C. R., K. L. Bowman, M. D. Tabatchnick, and C. H. Lamborg. 2011. Storage bottle material and cleaning for determination of total mercury in seawater. Limnol. Oceanogr. Methods 9:426-431 [doi:10.4319/lom.2011.9.426].

———, and K. L. Bowman. 2012. Vertical methylmercury distribution in the subtropical North Pacific Ocean. Mar. Chem. 132-133:77-82 [doi:10.1016/j.marchem.2012.02.005].

Horvat, M., L. Liang, and N. S. Bloom. 1993. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples: Part II, Water. Anal. Chim. Acta 282:153-168 [doi:10.1016/0003-2670(93)80364-Q].

Lamborg, C. H., C. R. Hammerschmidt, G. A. Gill, R. P. Mason, and S. Gichuki. 2012. An intercomparison of procedures for the determination of total mercury in seawater and recommendations regarding mercury speciation during GEOTRACES cruises. Limnol. Oceanogr. Methods 10:90-100 [doi:10.4319/lom.2012.10.90].

Lehnherr, I., V. L. St. Louis, H. Hintelmann, and J. L. Kirk. 2011. Methylation of inorganic mercury in polar marine waters. Nat. Geo. 4:298-302 [doi:10.1038/ngeo1134].

Mason, R. P., and W. F. Fitzgerald. 1993. The distribution and biogeochemical cycling of mercury in the equatorial Pacific Ocean. Deep-Sea Res. I 40:1897-1924 [doi:10.1016/0967-0637(93)90037-4].

———, K. P. Rolfhus, and W. F. Fitzgerald. 1995. Methylated and elemental mercury cycling in surface and deep ocean waters of the North Atlantic. Water Air Soil Pollut. 80:665-677 [doi:10.1007/BF01189719].

Monperrus, M., E. Tessier, D. Amouroux, A. Leynaert, P. Huonnic, and O. F. X. Donard. 2007. Mercury methylation, demethylation and reduction rates in coastal and marine surface waters of the Mediterranean Sea. Mar. Chem. 107:49-63 [doi:10.1016/j.marchem.2007.01.018].

Parker, J. L., and N. S. Bloom. 2005. Preservation and storage techniques for low-level aqueous mercury speciation. Sci. Total. Environ. 337:253-363 [doi:10.1016/j.scitotenv.2004.07.006].

Scheuhammer, A. M., M. W. Meyer, M. B. Sandheinrich, and M. W. Murray. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36(1), 12-19. [doi:10.1579/0044-7447(2007)36[12:EOEMOT]2.0.CO;2]

Sunderland, E. M., D. P. Krabbenhoft, J. W. Moreau, S. A. Strode,

and W. M. Landing. 2009. Mercury sources, distribution, and bioavailability in the North Pacific Ocean: Insights from data and models. Global Biogeochem. Cycles 23:GB2010.

Tekran Instruments Corporation. 2011. Series 2700 Methyl Mercury Analysis System: Analytical Guides. Rev: 1.10.

US EPA (USEPA). 2001. Method 1630: methyl mercury in water by distillation, aqueous ethylation, purge and trap, and cold vapor atomic spectrometry. Washington, DC.

Submitted 24 June 2013
Revised 2 December 2013
Accepted 16 December 2013